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The Effect of Copper on the Growth of Bacteria Isolated from Marine Environments

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ABSTRACT

The use of copper may prove feasible for the control of catastrophic fish mortalities associated with blooms of *Gymnodinium brevis*. Prerequisite is knowledge of the effect that relatively high concentrations of copper may have on the marine community as a whole. In this study the effect of copper on the growth of 145 marine bacteria was measured. All of the bacteria grew in a medium containing copper in concentrations up to 0.25 mg/L. This concentration was compared to the *in vivo* (0.2 mg/L) and *in vitro* (0.1 mg/L) lethal dose of copper for *G. brevis*. In addition, the growth of approximately 4 per cent of the bacteria was stimulated by this concentration of copper.

INTRODUCTION

During the latter part of 1955, red tide outbreaks associated with *Gymnodinium brevis* were reported along the Gulf coast of Mexico and along the southeast coast of Texas (Wilson and Ray 1956). These blooms were accompanied by extensive kills involving millions of pounds of commercial and game fish, paralleling the destructiveness of Florida's 1946 outbreak (Gunter *et al.* 1948, Galtsoff 1948). In all of these instances *G. brevis* (Davis 1948) was identified and found in large numbers. Other red tide outbreaks have been reported (Feinstein *et al.* 1955, Lackey and Hynes 1955).

Recently *G. brevis* was isolated and is being maintained in pure culture. Results of laboratory experiments by Dr. S. Ray and Mr. W. Wilson[1] with unialgal and pure cultures demonstrated that concentrations of *G. brevis* comparable to those of the Florida red tide were toxic to fish.

This paper reports on one aspect of a major project concerning red tide control measures presently in progress by the Gulf Fishery Investigations of the U. S. Fish and Wildlife Service. The study was prompted by the proposal that treatment of possible foci of infection with heavy metals may prove practical for the control of catastrophic blooms of *G. brevis*. Prior to large-scale field operations, it was decided to investigate the possibility of adverse effects of heavy metals on organisms other than *G. brevis*. Copper was selected because initial studies by Mr. Wilson showed that *G. brevis* is extremely sensitive to concentrations of copper only slightly above those normally present in regions along the west Florida coast (Mr. J. Bell[1]).

Copper has been used extensively for the control of algal blooms. Whipple (1948) cited Kellerman who studied the effect of copper sulfate upon fresh-water fish. It was shown that concentrations used for the destruction of microscopic organisms were close to the doses that would kill fish. A list was compiled by Chow and Thompson (1952) of the copper content of sea water found by various investigators in a variety of localities. Riley (1937) found a range of 0.001–0.015 mg/L off the mouth of the Mississippi River. At one of his stations a range of 0.02–0.025 mg/L was found. Atkins (1953) reported a seasonal variation of copper in surface waters of the English Channel ranging from 0.0015 mg/L in early autumn to 0.025 mg/L in winter. Higher values for Long Island Sound (Prytherch 1934, Galtsoff 1943) were attributed by Chow and Thompson (1954) to discharges of industrial wastes.

The effect of copper on various organisms involved in marine fouling has been re-

[1] We should like to thank Dr. S. Ray and Mr. W. Wilson of this laboratory and Mr. J. Bell of our field station in Naples, Florida, for making this information available prior to publication.

viewed (Woods Hole Oceanogr. Inst. 1952). Harvey (1955) stated that the concentration of cupric ion which is poisonous to marine plants and animals was approximately 1.0 mg/L. However, the effective algicidal dose of copper sulfate *in vivo* is dependent on the chemical and physical properties of the water to which it is added. Soluble and insoluble components will effectively chelate copper and hence decrease its effectiveness. Harvey (1955) stated that when added to sea water ethylene-diamine-tetra-acetate would reduce the poisonous effect of copper on algae. In experiments with unialgal cultures of *G. brevis*, Mr. Wilson found that EDTA reduced the toxic effect of otherwise lethal doses of copper.

This study reports on the effect of copper on the growth of bacteria which were isolated from marine samples. The physiological and biochemical properties of the isolates and the effect of heavy metals on such activities will be the subject of another paper.

METHODS AND MATERIALS

Bacteria were isolated from mud and water samples (Table 1) taken from a lagoon (Lagoon 2 and 3), from mullet (*Mugil* sp.) intestinal contents (Mullet a, b, and c), from the slime of an eel (*Mystriophis* sp.), from laboratory tanks of unialgal cultures of *G. brevis* (Tank B_1 and B_5), and from the southeast coast of Texas during a *G. brevis* bloom (Tex. Red Tide). Other cultures (Florida) were obtained from the collection of Dr. S. Ray. Most of Dr. Ray's cultures were isolated from samples taken along the west coast of Florida.

TABLE 1. *Source and numbers of bacterial cultures which would grow in and below the concentration of copper indicated*

Source of culture	Cu, mg/L									
	0	0.25	1.3	3.1	7.6	12.7	20.4	25.4	35.6	50.9
Mullet a					1		1	2	2	
Mullet b					1	1		1	3	4
Mullet c					3	1	5	3	5	4
Lagoon 2		1	1	2	1	4	5		1	
Lagoon 3				7	7	9	1	5	3	8
Florida				1	1	5	2	5	2	3
Texas Red Tide						2		1		
Eel						1	1			
Tank B_1				1		1		3		
Tank B_5		1		2	2	3	1	3	6	6

Samples were plated by the dilution plate count technique or streaked directly onto Medium A of the following composition: trypticase, peptone, and yeast extract, 0.005 per cent each; sodium acetate, 0.01 per cent; Bacto agar, 1.2 per cent; and made to volume with aged Gulf water collected 50 miles off the southwest coast of Florida. The final pH of the medium was 7.4–7.8 after autoclaving at 121°C for 15 min. Plates were incubated for 2–15 days at 20–25°C.

Isolated colonies were picked and restreaked several times to ensure culture purity. Stock cultures were maintained at 20–25°C in screw-cap tubes on the following medium: trypticase, peptone, yeast extract, 0.0075 per cent each; sodium acetate, 0.01 per cent; Bacto agar, 0.2 per cent; and made to volume with aged Gulf water.

Bacterial types were tentatively determined according to the following criteria: nitrate reduction, degradation of chitin and cellulose, catalase reaction, liquefaction of gelatin, anaerobic growth, growth patterns, temperature range, Gram stain, phase-microscopic morphology and motility, and source of isolation. Only those isolates which differed from one another in one or more of these characteristics are reported here. Thus each culture represented a major morphological and physiological category or a distinct variant. No attempt was made to assign generic or specific names to the organisms.

The effect of $CuSO_4 \cdot 5H_2O$ on growth of the bacteria was tested in Medium A minus the agar. Copper sulfate was added to give the following final concentrations of copper: 0.0, 0.25, 1.3, 3.1, 7.6, 12.7, 20.4, 25.4, 35.6, and 50.9 mg/L. The pH of each batch of medium was adjusted with 1 N NaOH to pH 7.4–7.6. This was necessary because the addition of the larger amounts of copper lowered the pH considerably. Media were tubed in 5 ml quantities in screw-cap test tubes and autoclaved at 121°C for 15 min.

Inoculum was prepared by diluting one drop of a 24–48 hr culture, grown in Medium A minus the agar, in 5 ml of the same

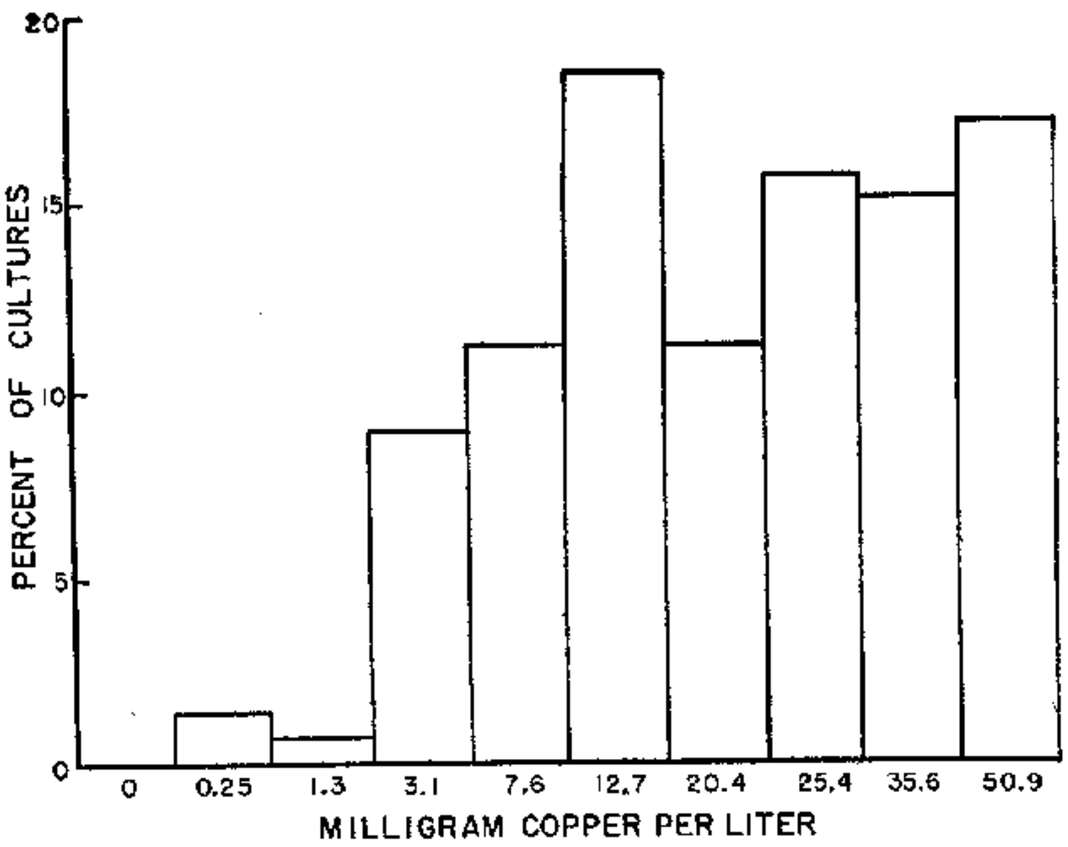

FIG. 1. Per cent of cultures that showed minimal growth in concentration of copper indicated.

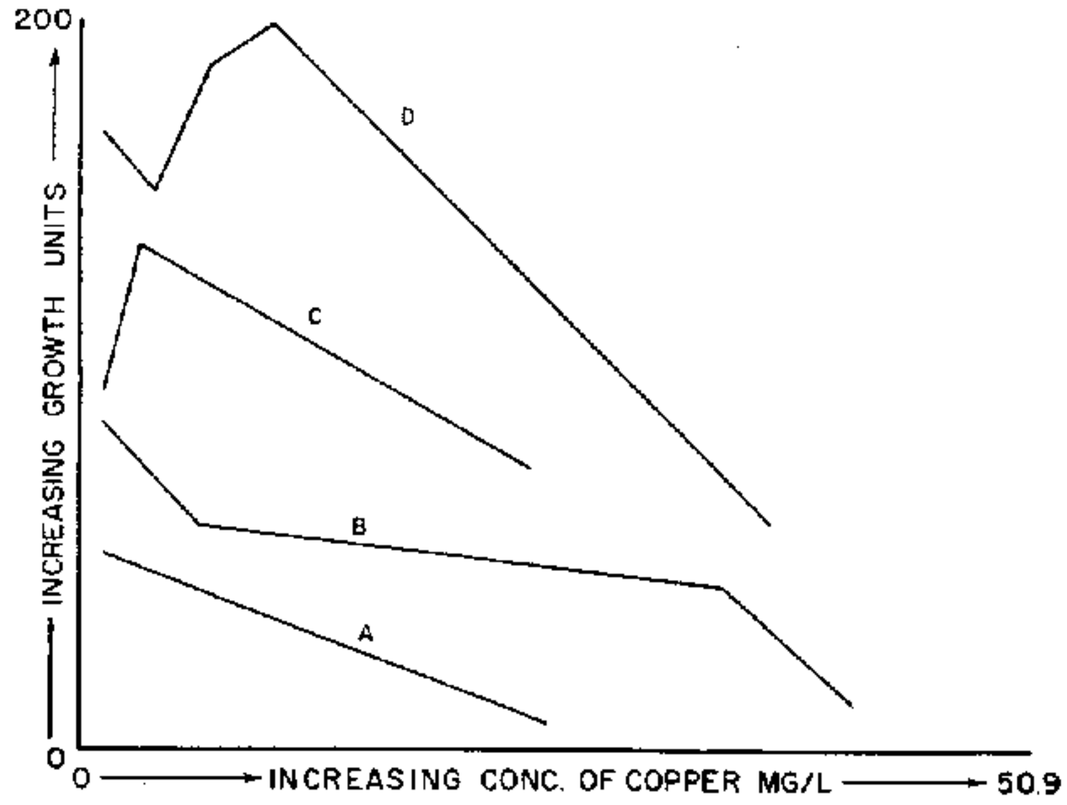

FIG. 2. Typical growth responses of cultures to increasing copper concentrations.

medium. One drop of this suspension was used per tube.

Cultures were incubated at 24°C for 4 days unless otherwise indicated. Growth was measured turbidimetrically with a Klett-Summerson colorimeter using a number 42 filter.

RESULTS

The effect of copper on the growth of 145 cultures is given in Table 1. The source of each isolate and the number of cultures which grew in the presence of and below the indicated concentrations of copper are shown. Medium A minus the agar supported growth of all the cultures. Except for 3 cultures which required 8 days' incubation, turbidimetric measurements were recorded after 4 days. These 3 slow-growing cultures were sensitive to copper concentrations exceeding 1.3 mg/L, whereas the remainder grew within a range of 3.1–50.9 mg/L. Twenty-five grew in the highest concentrations of copper used. As shown in Figure 1, the frequency of occurrence of cultures tolerant to copper increased with increasing concentrations of copper.

Typical growth responses of the cultures to increasing concentrations of copper are shown in Figure 2. Curve *A* represented 79 per cent of the cultures. The slope ranged from a maximum for those cultures which were inhibited by the lower copper concentrations to a minimum for those cultures which were relatively unaffected by the higher quantities. One culture grew equally well at all copper concentrations. Curve *B* was representative of 7 per cent of the cultures. The predominant growth characteristics were a slight inhibition at low copper concentrations, followed by a plateau, and then an inhibition at high copper concentrations. Curve *C* represented 4 per cent of the cultures. The growth of members of this group was stimulated by small additions of copper. Curve *D* is representative of 10 per cent of the cultures. The supposedly stimulatory effect at the lower copper levels was attributed to increased pigment production.

DISCUSSION

The use of copper may be practical for the control of catastrophic fish mortalities associated with blooms of *G. brevis*. This type of control requires some knowledge of the effect that relatively high concentrations of copper may have on the marine community as a whole.

In these experiments concentrations of copper up to 0.25 mg/L did not inhibit the initial growth of any of the bacteria that were tested. Except for 3 normally slow-growing cultures, all of them grew in the presence of 3.1 mg/L. Mr. Wilson demonstrated that *G. brevis* is killed by 0.05–0.1 mg/L. The addition of 7.6 mg/L allowed the growth of 89 per cent of the isolates; 12.7 mg/L allowed growth of 78 per cent.

Provided that the bacteria tested are representative of areas susceptible to *G. brevis*

blooms, the concentration of copper could be effectively increased to 0.25 mg/L without bactericidal effects. Harvey (1955) estimated that the concentration of cupric ion which is toxic to marine plants and animals was approximately 1.0 mg/L. This concentration is approximately 10 times higher than the lethal dose for *G. brevis* in laboratory experiments and 5 times the minimum lethal dose (MLD) established by Mr. Bell in red tide areas along the west coast of Florida. At copper concentrations of 1.3 mg/L, approximately 1.4 per cent of the bacteria screened did not grow. Waksman *et al.* (1943) reported that concentrations of copper up to 10 mg/L did not affect the number of bacteria found in water taken near the dock of the Oceanographic Institution at Woods Hole. Thus, it appears that an *in vivo* MLD of 1.3 mg/L would not materially affect the growth of bacterial populations. In addition, this concentration of copper stimulated the growth of 4 per cent of the cultures as shown in Figure 2, Curve *C*.

SUMMARY

Bacteria were isolated from a number of marine samples. On the basis of differential morphological and physiological properties, 145 different cultures were selected and the effect of increasing copper concentrations on their growth was measured.

Growth patterns of the cultures as affected by copper were divided into four representative groups which are described.

All of the cultures grew in a basal medium containing a copper concentration of 0.25 mg/L. Seventeen per cent tolerated up to 50.9 mg/L which was the highest concentration of copper tested. These values were compared with the *in vitro* lethal concentration (0.1 mg/L) for *Gymnodinium brevis* and the estimated minimum lethal dose (MLD) *in vivo* (0.2 mg/L). At the MLD level, all of the bacteria grew and the growth of approximately 4 per cent was stimulated.

Considering that the bacteria tested were representative of areas susceptible to *G. brevis* blooms, an *in vivo* MLD of 1.3 mg/L would not materially interfere with the growth of these bacteria and may be beneficial to some.

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